18.08.14

Overlap extension PCR of
-split GFP-N+hog1+Kan P8
-rLuc-pbs2+Kan P9
-split rLucN-pbs2+Kan P10

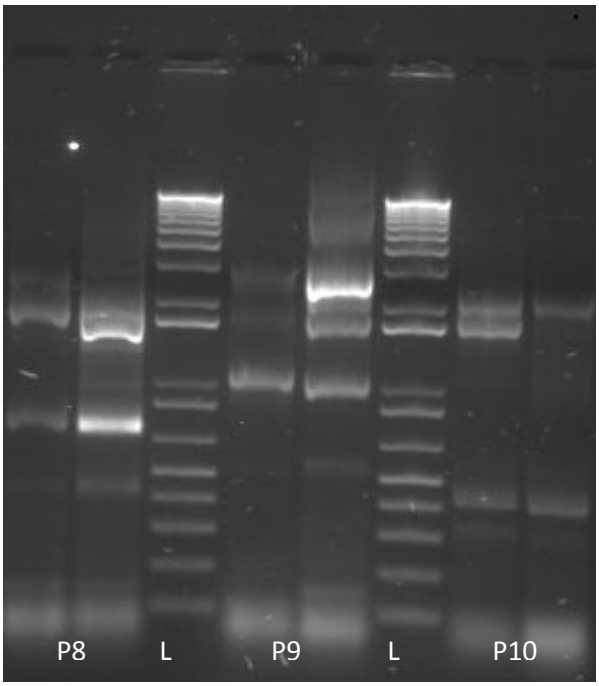
25µL reactions
Equimolar template ratios: we used 200 ng of the longest template, and estimated the necessary amount of the shortest template with its size.

In µL	Master Mix (GC buffer)	Template 1	Template 2 (100ng)	Water	Primers*
P8: P4+P7	5.75	P4: 1.6	P7: 4	8.75	2.5 each (p12/p2)
P9: P1+P5	5.75	P1: 1.1	P5: 2	11.2	2.5 each (p12/p2)
P10: P2+P6	5.75	P2: 0.8	P6: 4	9.5	2.5 each (p12/p2)
Duplicates were made					

*Add primers after the first program (after fusion of the templates).

Thermocycler programs :
P8,9,10 : 15 cycles at 63°C for overlap, add primers, then 15 cycles at 70°C annealing. Always 2min30 of extension time at 72°C.

Results:



The fusion of all the fragments seems to have proceeded (thanks to 63°C annealing temperature, the primers of the previous PCR that were not washed away could not interfere), but the 70°C temperature chosen for the amplification doesn't work for P8 and P10. Next time: DMSO duplicates and annealing of 72°C.

It isn't impossible that 200ng of the largest template is way too high for a 25ul reaction: next time, only 50ng.

Overlap extension PCR of
-split GFP-N+hog1+Kan P8
-split rLucN-pbs2+Kan P10

25µL reactions
Equimolar template ratios: we used 50 ng of the longest template, and estimated the necessary amount of the shortest template with its size.

In µL	Master Mix (GC buffer)	Template 1	Template 2 (100ng)	Water	Primers*
P8: P4+P7	5.75	P4: 2.76	P7: 2	9.49	2.5 each (p12/p2)
P10: P2+P6	5.75	P2: 0.4	P6: 2	11.1	2.5 each (p12/p2)
Duplicates with DMSO were made -> Add 0.75ul DMSO and 0.75ul of water					

*Add primers after the first program (after fusion of the templates).

Thermocycler programs :
P8,10 : 15 cycles at 63°C for overlap, add primers, then 15 cycles at 72°C annealing. Always 2min30 of extension time at 72°C.

Results: